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Short communication

Physical exercise affects the epigenetic programming of rat brain and modulates the adaptive response evoked by repeated restraint stress

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HIGHLIGHTS

- Physical exercise increase the DNA methylation profile of rat's hypothalamus.
- Physical exercise modulates the epigenetic responses evoked by RRS.
- Decreased expression of *Dnmt1* gene is evoked by repeated stress in exercised rats.

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ABSTRACT

Epigenetics has recently been linked to molecular adaptive responses evoked by physical exercise and stress. Herein we evaluated the effects of physical exercise on global DNA methylation and expression of the *Dnmt1* gene in the rat brain and also verified its potential to modulate responses evoked by repeated restraint stress (RRS). Wistar rats were classified into the following experimental groups: (1) physically active (EX): animals submitted to swimming during postnatal days 53–78 (PND); (2) stress (ST): animals submitted to RRS during 75–79PND; (3) exercise-stress (EX-ST): animals submitted to swimming during 53–78PND and to RRS during 75–79PND, and (4) control (CTL): animals that were not submitted to intervention. Samples from the hippocampus, cortex and hypothalamus were obtained at 79PND. The global DNA methylation profile was assessed using an ELISA-based method and the expression of *Dnmt1* was evaluated by real-time PCR. Significantly increased methylation was observed in the hypothalamus of animals from the EX group in comparison to CTL. Comparative analysis involving the EX-ST and ST groups revealed increased global DNA methylation in the hippocampus, cortex, and hypothalamus of EX-ST, indicating the potential of physical exercise in modulating the responses evoked by RRS. Furthermore, decreased expression of the *Dnmt1* gene was observed in the hippocampus and hypothalamus of animals from the EX-ST group. In summary, our data indicate that physical exercise affects DNA methylation of the hypothalamus and might modulate epigenetic responses evoked by RRS in the hippocampus, cortex, and hypothalamus.

Epigenetic mechanisms have been increasingly associated with molecular pathways related to biological responses evoked by physical exercise and stress [1,2].

Experimental studies have shown that physical exercise induces an increase in adaptive responses of the HPA axis and improves cognitive responses to psychological stress [3]. Furthermore, physical exercise has been considered an important environmental stimulus for altering the transcriptional machinery of genes involved in brain function [4,5].

Regular physical exercise is considered to be a potential prophylactic and therapeutic recommendation for various disorders due to its impact on the improvement of cognitive function [6,7], reduction of anxiety and depression [8] and the ability to protect the brain against neurodegenerative disorders [9,10].

The use of validated experimental approaches is a suitable alternative for the evaluation of the impact of environmental exposures such as the practice of physical exercise and behavioral stress on brain cells. In this context, rat models of restraint stress and

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swimming have been considered important methodological approaches for the evaluation of the effects of behavioral stress and physical exercise, respectively [11,12].

Recently, our group reported on the association of significant changes in the global DNA methylation profile of brain areas, such as the hippocampus, cortex and PAG, with the adaptive responses evoked by acute restraint stress [13]. Furthermore, we demonstrated the potential of practicing physical exercise in modulating epigenetic changes evoked by acute stress [13].

Considering that this previous report lacks information on the impact of physical exercise on epigenetic patterns of non-stressed animals, or the effect of physical exercise in animals submitted to repeated stress, herein we focused on the identification of the impact of swimming training on global DNA methylation and on expression of the *Dnmt1* gene in the hippocampus, cortex and hypothalamus by comparing the methylation and expression profiles of sedentary and exercised animals. Furthermore, we assessed the potential of swimming for modulating the impact of repeated sessions of restraint stress (RRS) on the adaptive epigenetic responses detected in the aforementioned brain areas.

The experimental procedures were carried out following protocols approved by the local ethical review committee (CEUA No 14441.2013.18). All the experimental protocols were conducted in the morning period between 07:00 and 12:00 h in the light phase.

Non-related male Wistar rats (52 days old at the beginning of the experiments) were kept at room temperature (25 ± 1 °C, photoperiod of 12 h light/12 h dark) with water and food *ad libitum*, in the Central Animal House of the University of Londrina.

The animals were classified into four groups containing 4–10 animals each: (1) exercise group (EX): animals that were submitted to physical exercise (swimming for 60 min/day) from the 53rd postnatal day (PND) to 78PND; (2) stress group (ST): animals that were submitted to repeated restraint stress during 75–79PND; (3) exercise and stress group (EX-ST): animals that were submitted to physical exercise (swimming for 60 min/day) during 53–78PND and also to repeated restraint stress from 75–79PND; and (4) control group (CTL): animals that were kept in the same room conditions but were not submitted to swimming or physical exercise protocols. The animals from all experimental groups were killed by decapitation at 79PND for sample collection.

The animals in the ST and ST-EX groups were transported in the morning period (7:00 to 09:00) to the experimental room in their home cages and allowed to adapt to this environment for at least 60 min.

Following this period, the animals were subjected to the restraint stress protocol, being placed in a metal cylinder 6.5 cm in diameter and 15 cm long with holes that allow ventilation, where they remained fixed and were kept enclosed for 60 min.

Animals in the EX and EX-ST group were subjected to physical training (swimming), without stimulus or tasks, according to Martins-Pinge et al. [12]. The swimming sessions were performed in a glass tank filled with lukewarm water (31 ± 1 °C) with a 4000 cm^2 surface area and 60 cm depth.

The training consisted of 4 weeks (20 sessions) of swimming being done five days a week and 60 min/day. During the first week, the training was graded, starting with 15 min on the first day, 30 min on the second day, and 45 min on the third day, for adaptation to the training process. From the fourth day on, each session consisted of 60 min of swimming until 78PND. The animals were assisted during the entire swimming sessions in order to avoid passive floatation and minimize bias related to the differential intensity of physical exercise. After each swimming session, the animals were dried with a towel and kept for a readjustment period together in a box, and then returned to their individual cages.

Samples were collected in the morning (8:00 to 12:00) to avoid interference of the circadian cycle on the biological variables. The animals were killed by decapitation and blood samples were collected from the trunk.

The brain was rapidly removed from the skull and a sample of the hypothalamus, frontal cortex area (approximately 2 mm including the cingulate cortex area and excluding the corpus callosum), and the hippocampus were obtained immediately. Brain regions were sectioned according to the anatomical atlas of Paxinos and Watson [14] and tissues were stored at −80 °C.

Genomic DNA was obtained by a standard salting out protocol [15]. Evaluation of the purity and concentration of DNA was performed by analysis of absorbance in a spectrophotometer (NanoDrop ND-2000 – Thermo Scientific) at 260 nm and 280 nm.

The global DNA methylation profile was evaluated by dosage (percentage) of methyl groups (CH_3) using the Imprint Methylated DNA Quantification Kit (Sigma–Aldrich®) according to the manufacturer's recommendations and as previously described [11,12]. Briefly, the methylation status of each sample was calculated as the amount of methylated cytosine in the sample (5 mC) relative to global cytidine (5mC + dC) in a positive control (100% methylated) that had been previously methylated and a no template control sample (0% methylated) using absorbance readings at 450 nm and following the formula: (A450sample-A450NTC)/(A450met-A450NTC) × 100 [13,16]. All samples were analyzed in triplicate.

RNA samples were obtained using Trizol (Invitrogen®), and reverse transcription was performed using the High Capacity Kit (Applied Biosystems®, Foster City, CA, USA) according to the manufacturer's recommendations. Expression levels of *Dnmt1* genes were evaluated by Real-Time PCR (Applied Biosystems®, Foster City, CA, USA). Amplifications were obtained using on demand Taqman probes (Applied Biosystems®, Foster City, CA, USA) for the *Dnmt1* gene (Assay ID Rn00709664_M1). For normalization of the difference in the amount of cDNA we used the *Gapdh* gene (Assay ID Rn01775763_G1) as an endogenous control. Experiments were performed in triplicate.

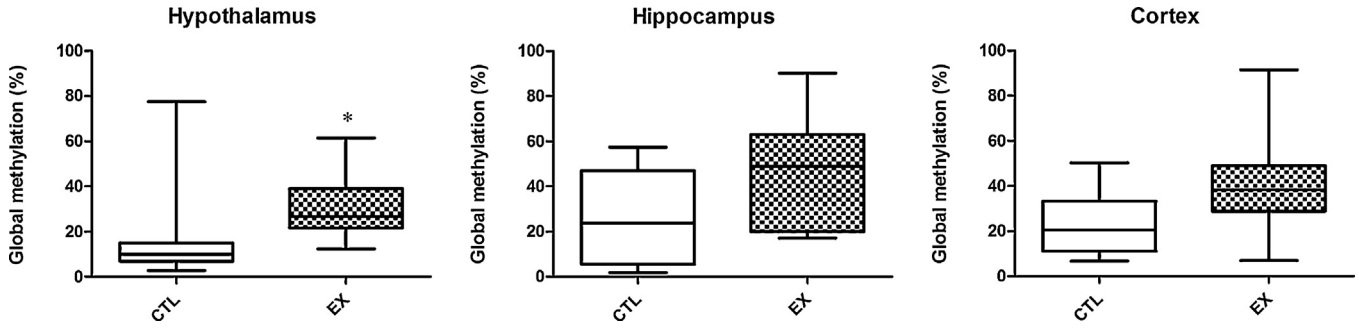

Fig. 1. Graphic representation of the impact of physical exercise on the global DNA methylation profile of the Hypothalamus, Cortex and Hippocampus of rats. CTL: control group; EX: exercise group. *$P < 0.05$. Mann–Whitney test.